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Regional Localisation of Two Non-Specific X-Linked Mental Retardation Genes (*MRX30* and *MRX31*)

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Two genes responsible for X-linked mental retardation have been localised by linkage analysis. *MRX30* maps to a 28 cM region flanked by the loci *DXS990* (Xq21.3) and *DXS424* (Xq24). A significant multipoint lod score of 2.78 was detected between the loci *DXS1120* and *DXS456*. *MRX31* maps to a 12 cM region that spans the centromere from *DXS1126* (Xp11.23) to *DXS1124* (Xq13.3). Significant two-point lod scores, at a recombination fraction of zero, were obtained with the loci *DXS991* (Zmax = 2.06), *AR* (Zmax = 3.44), *PGK1P1* (Zmax = 2.06) and *DXS453* (Zmax = 3.31). The *MRX30* localisation overlaps that of *MRX8, 13, 20* and *26* and defines the position of a new MRX gene on the basis of a set of non-overlapping regional localisations. The *MRX31* localisation overlaps the localisations of many of the pericentromeric MRX loci *(MRX 1, 4, 5, 7, 8, 9, 12, 13, 14, 15, 17, 20, 22* and *26)*. There are now at least 8 distinct loci associated with non-specific mental retardation on the X chromosome defined, in order from pter to qter, by localisation for *MRX24, MRX2, MRX10, MRX1, MRX30, MRX27, FRAXE* and *MRX3*.

KEY WORDS: X-linked mental retardation, linkage, MRX30, MRX31

INTRODUCTION

X-linked mental retardation (XLMR) can be classified as either syndromal or non-specific, depending on the presence or absence of any other manifestations accompanying the retardation. XLMR is relatively common, with an estimated frequency of 1/600 male births [Herbst and Miller, 1980]. The fragile X syndrome accounts for 25–40% of this mental retardation [Sutherland and Hecht, 1985]. Non-specific forms of XLMR are allocated a progressive number preceeded by the symbol MRX, provided that the disease gene can be linked to one or more markers with a statistically significant lod score of at least 2 [Ott, 1991; Mulley et al., 1992]. It has been estimated that there are 7–19 MRX genes [Herbst and Miller, 1980]. Subsequently the fragile X syndrome and Renpenning syndrome were dissected out of this group by clinical geneticists, with the aid of a molecular test in the case of the fragile X syndrome [Oberlé et al., 1991; Yu et al., 1991]. One of the remaining non-specific types of mental retardation, FRAXE mental retardation, is now also identifiable by a molecular test [Knight et al., 1993].

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MATERIALS AND METHODS

MRX30 Family

The 4 generation MRX30 pedigree is shown in Figure 1a. This family came to light when III-2 asked for genetic counselling because of the mental retardation present in several males in her family.

Her brother (III-1) had been born at term after a normal pregnancy with a spontaneous vertex delivery. Birth weight was 3.25 kg. Feeding was difficult and he thrived poorly (Table I, Fig. 2a). His motor development was slow and significant delay was recognised by age 2 years. He repeated kindergarten and had special schooling: his IQ was measured at 63. He was described as restless and hyperactive. After school he spent time in a sheltered workshop and then got episodic, casual employment in the open work force; he lived at home with his mother. On examination at age 31 years (Table I; Fig. 2a) both his height and head circumference were on the first centile. No physical abnormalities were found; his mean ear length was 60 mm and mean testicular volume 18 ml.

The clinical histories of III-4 and III-9 were very similar though with less feeding difficulties. The certificates admitting II-3 to institutional care as a teenager in 1944 told the same story, but, in addition, there had been physical violence towards his mother. Physical examination of II-3 and III-9 showed no abnormalities except mild microcephaly in II-3. Chromosome studies of II-3 and III-1 were normal with no evidence of the

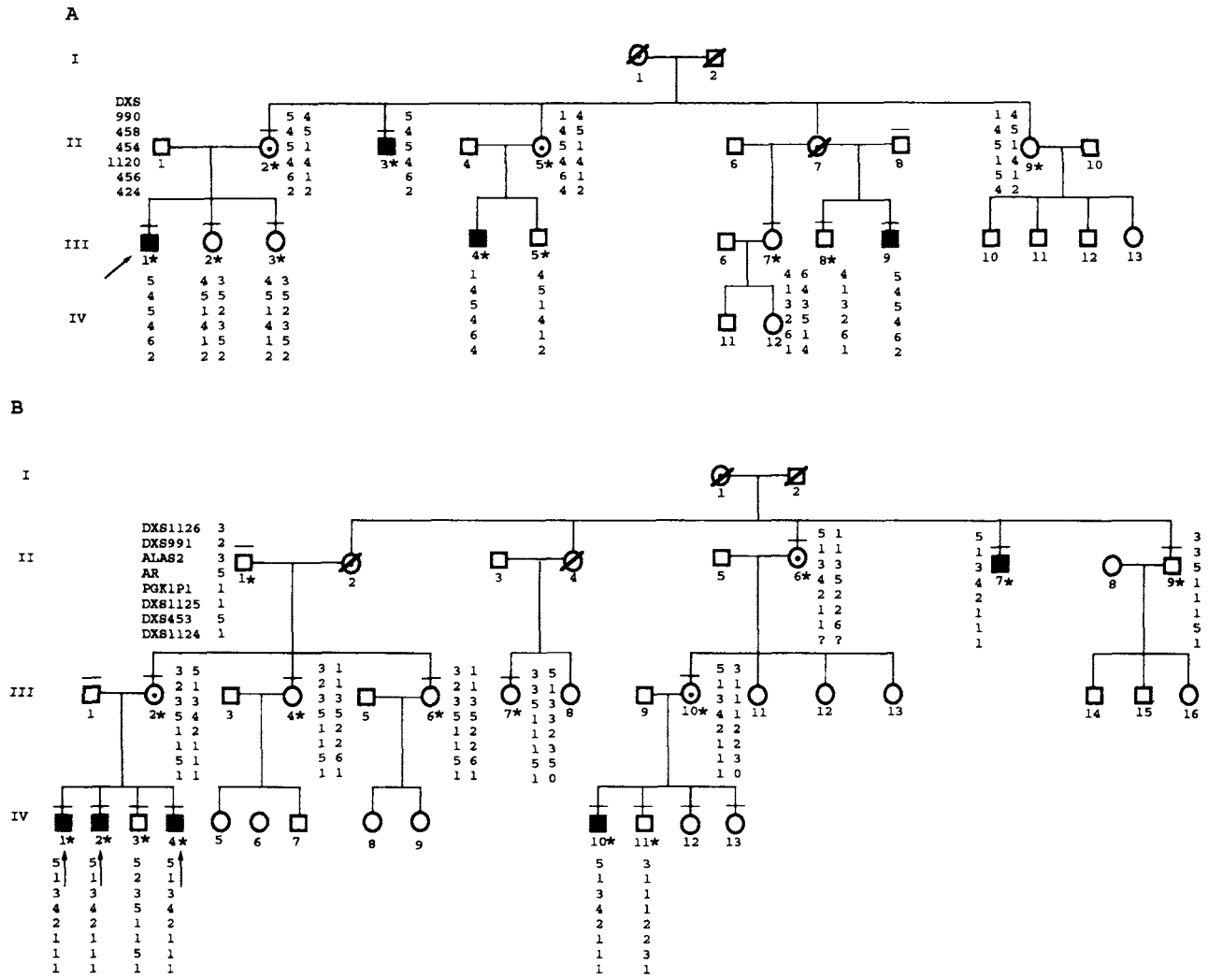

Fig. 1. **A:** MRX30 pedigree. **B:** MRX31 pedigree. →, propositus; ■, male with mental retardation; ⊙, obligate carrier; ⊘ deceased; —, personally examined; *, DNA available and analysed.

fragile X syndrome: DNA studies of III-1 excluded fragile X syndrome.

MRX31 Family

The 4 generation MRX31 pedigree is shown in Figure 1b. The parents (III-1, III-2) brought 3 of their sons (IV-1, IV-2 and IV-4) to the clinic seeking further understanding of their slow development. The family history showed that there was a similarly affected maternal cousin (IV-10) and great maternal uncle (II-7) suggesting X linkage.

All 3 brothers and their cousin (IV-10) were born without assistance at or near term after normal pregnancies: birth weight ranged from 3.1 to 3.94 kg (Table I). There were no feeding difficulties. Gross motor development of IV-1 and 2 was normal but both were slow to start speaking: global developmental delay was recognised at 4 years in IV-1 and 2 years in IV-2. By clinical observation and the mother's reports both seemed to have moderate intellectual handicap. IV-4 and IV-10 were said to be hypotonic as young children. IV-10 had been assessed (Stanford-Binet) at 6 years and was said to have an IQ "in the range 64 to 74"; IV-4 was at about the same level, or slightly less. On examination (Fig. 2b, Table 1) no major abnormalities were found. IV-1 had large ears (length of right ear, 65 mm) and a facial appearance suggesting the fragile X syndrome. IV-2 had a strabismus and wore glasses. All 3 affected brothers had a diastema between the upper central incisors as did their unaffected brother (IV-3) and their cousin IV-10 who also had a single palmar crease on one hand. II-7 had worked on a dairy farm for most of his adult life; he was illiterate and had lived with his mother and then in a nursing home. He could carry on a simple conversation; no physical abnormalities were found. Metabolic screens and chromosome studies had been carried out on IV-1 and IV-2; these were normal with no evidence of the fragile X.

TABLE I. Some Physical Measurements of Various Relatives

Family	Subject	Place in pedigree	Age years-months	Birthweight kg (%)	Height cm (%)	Weight kg (%)	OFC cm (%)
MRX30	PH	III-9	21	—	170 (15)	—	54 (25)
	JH[a]	III-8	24	—	177 (50)	—	56 (50)
	MG	III-4	29	—	172 (15)	—	55 (40)
	MT	III-1	31	3.25 (50)	167 (10)	53.8 (5)	52 (1)
	GW	II-3	67	—	165 (3)	—	52 (1)
MRX31	SZ	IV-4	5-3	3.65 (75)	113 (60)	19.3 (50)	51 (50)
	PB[a]	IV-11	5-9	3.31 (50)	114 (40)	18.5 (25)	52 (60)
	TB	IV-10	7-6	3.10 (25)	126 (55)	21.3 (10)	51 (30)
	AZ[a]	IV-3	6-7	4.10 (95)	115 (10)	20.6 (25)	54 (90)
	CZ	IV-2	10-7	3.94 (90)	151 (95)	44.8 (95)	53 (50)
	JZ	IV-1	12-6	3.65 (75)	164 (95)	60.8 (95)	55 (75)
	RH	II-7	65	—	174 (40)	77.0 (75)	55 (40)

[a] Unaffected; % = approximate centile for age; OFC = occipito-frontal (head) circumference.

DNA Analysis

Genomic DNA was extracted from venous blood using standard procedures. Initially the families were screened using microsatellite markers spanning the X chromosome. Once linkage was found additional markers in the region were genotyped to narrow the localization as defined by loci exhibiting recombination with the disease gene. The markers used are given in Table II. The relative genetic positions of these markers were taken from Donnelly et al. [1994b], except for *DXS365* [Donnelly et al., 1994a] and *DXS1108* [Freije et al., 1992].

PCR was performed as previously described [Donnelly et al., 1994a], with the exception that most pairs of primers worked using a final $MgCl_2$ concentration of 1.5 mM, a final (non-radioactive) dNTP concentration of 200 μM and a final alpha-P^{32}-dCTP concentration of 2.5 μCi.

Linkage Analysis

Two point linkage analyses between each marker and the disease locus were performed using MLINK of the LINKAGE program package version 5.1 [Lathrop and Lalouel, 1984], assuming X-linked recessive inheritance. The frequency of the disease allele was arbitrarily chosen as 0.0001. Penetrance was set at 1.0 for males and 0.0 for carrier females. The number of alleles of each marker was "down-coded" to four and their population frequencies were assumed to be equal (i.e., 0.25 each).

For multipoint analysis of the MRX30 genotypes, the LINKMAP program of the LINKAGE package was used [Lathrop et al., 1984]. The loci used in this analysis are given in Table III. The disease locus was analysed relative to a "window" of four marker loci at a time that was moved along the map one locus at a time.

RESULTS

MRX30

Two-point linkage analysis of the data from the MRX30 family indicated that the locus most likely resides within a 28 cM region between the loci *DXS990* (Xq21.3) and *DXS424* (Xq24). None of the markers within this region were fully informative, which prevented the achievement of a significant lod score by two-point analysis (Table III). However, multipoint analysis, provided a significant multipoint lod score of 2.78 (Fig. 3) which corresponded with the theoretical maximum from two-point analysis of a fully informative marker. Recombination between the disease locus and both the proximal (*DXS990*) and distal (*DXS424*) loci was exhibited in the female carrier II-5.

Inspection of the pedigree of this family (Fig. 1a) prior to collection of blood samples suggested that the MRX gene might be unmappable. To obtain a significant lod score from this family the meioses leading to II-2, II-3, II-5 and II-7 needed to be rendered potentially informative by inference of the marker genotypes for I-1. II-2, II-5 and II-7 all received the MRX gene, hence their chromosomal segment within the immediate vicinity of the MRX gene would be derived from the same homologue of I-1 which would preclude inference of heterozygosity for tightly linked markers. This would remain the case if II-9 was a carrier, however her potential non-carrier status was high, given three normal sons. It was therefore considered likely that she received the wild-type MRX containing segment from the alternative homologue which would allow the inference of heterozygosity in I-1. This was in fact the case and II-9 was crucial for the successful mapping of MRX30 even though her carrier status was unknown at the commencement of this study.

MRX31

Two-point linkage analysis of the data from the MRX31 family showed that the localisation encompassed 12 cM from *DXS1126* (Xp11.23) to *DXS1124* (Xq13.3). Significant lod scores were obtained with the loci *DXS991* (Zmax = 2.06), AR (Zmax = 3.44), *PGK1P1* (Zmax = 2.06) and *DXS453* (Zmax = 3.31), all at recombination frequencies of zero (Table IV). Recombination of the disease locus with the proximal locus (*DXS1126*) and the distal locus (*DXS1124*) was exhibited in the unaffected males IV-3 and IV-11, respectively.

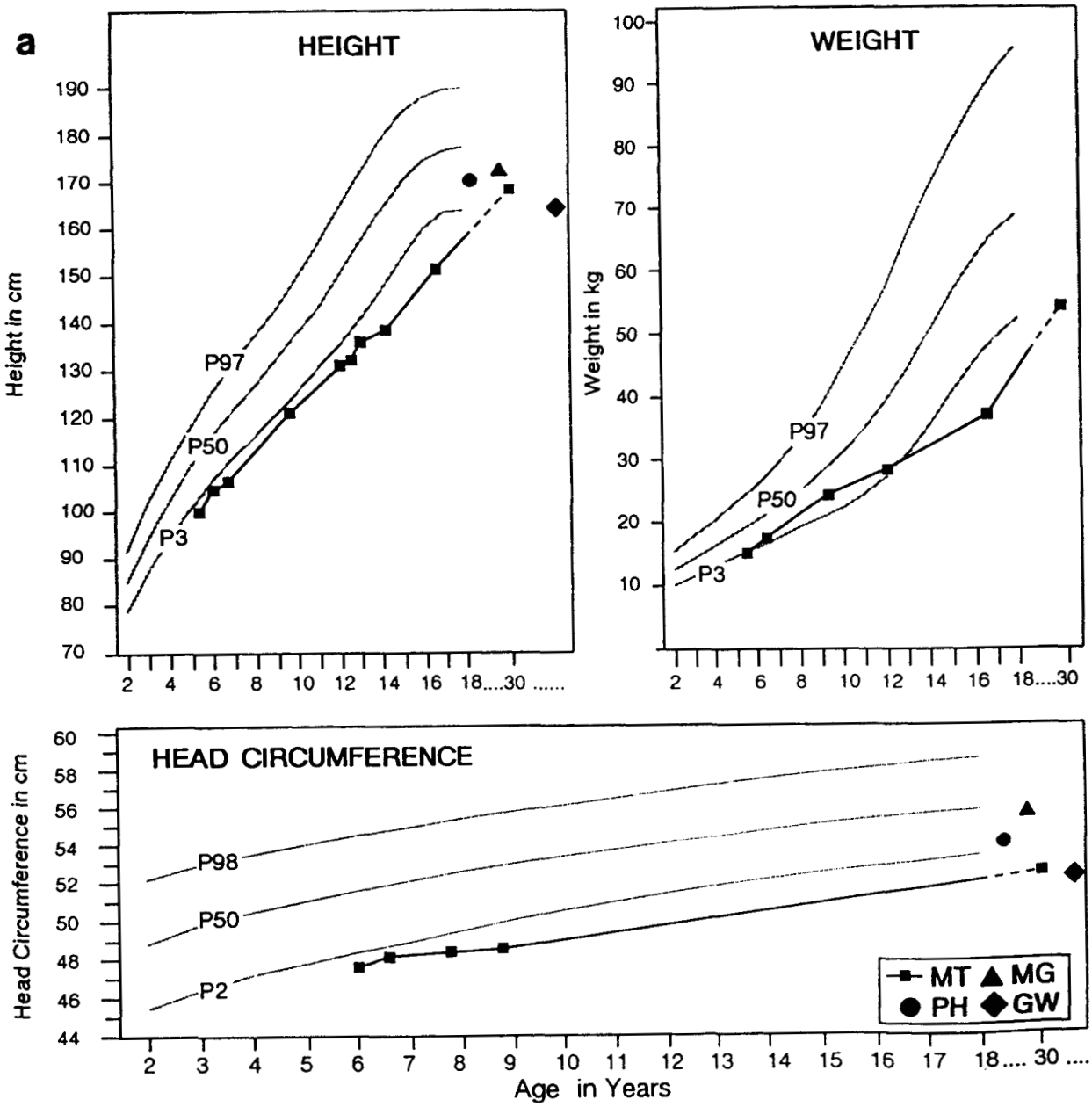

Fig. 2a. Growth measurements of the propositus (MT, III-1), through childhood and single measurements as adults of the other affected individuals (MG, III-4; PH, III-9; GW, II-3) of the MRX30 family.

DISCUSSION

Overall the two families differed from one another clinically in that the affected individuals in MRX30 had mild mental retardation with height and OFC below average whereas in MRX31 the mental retardation was moderate with height and OFC average or above. However, all the physical measurements were within the normal range except for the OFC in III-1 and II-3 from MRX30 which were on the first centile or 3 standard deviations below the mean. No single affected individual had specific physical or mental characteristics that would have allowed the diagnosis in the absence of the family history; indeed, without the family history III-1 and II-3 may well have been diagnosed as having primary microcephaly. In other words both families had non-specific XLMR.

The genes responsible for 53 of the 127 XLMR disorders have been regionally localised [Neri et al., 1994]. The *MRX30* and *MRX31* localisations overlap many of these. From a consideration of the MRX localisations [Gedeon et al., 1996], there are at least 8 distinct loci responsible for non-specific retardation on

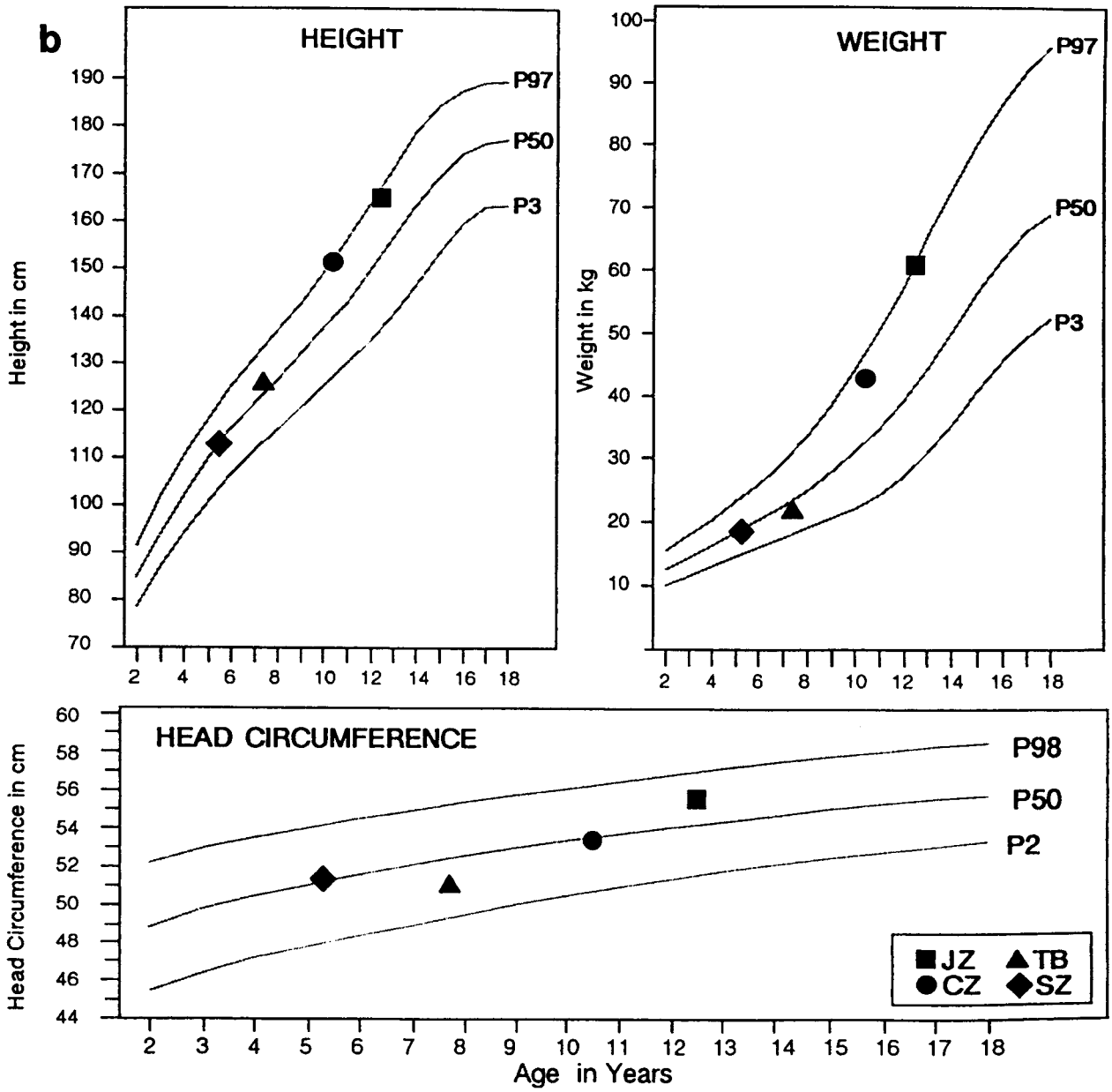

Fig. 2b. Single growth measurements in the 3 affected brothers (JZ, IV–1; CZ, IV–2; SZ, IV–4) and their maternal cousin (TB, IV-10) of the MRX31 family.

the X chromosome if FRAXE mental retardation is included. The other 7 regions from pter to qter are defined by *MRX24, MRX2, MRX10, MRX1, MRX30, MRX27* and *MRX3*. Thus, the *MRX30* localisation now reported has led to the delineation of a new region for MRX. This degree of heterogeneity prevents combining data from MRX families with overlapping regional localisations for the purpose of reducing regions for candidate genes.

To resolve whether or not overlapping regional localisations reflect a range of allelic mutations within one or a few genes or whether they are a consequence of mutations at many loci involved in brain development and function will require the cloning of candidate genes and the subsequent screening of the DNA or RNA of affected males for mutations [Mandel, 1994]. These candidate genes may be provided by the cloning of genes associated with chromosomal abnormalities of mentally retarded males or females. Deletions and translocations associated with mental retardation have been observed in Xp22.3 [Ballabio et al., 1989], Xq13.1 [Van der Maarel et al., 1994] and Xq21 [May et al., 1995]. An alternative approach is to screen any gene, known to map within the regional localisation,

TABLE II. Microsatellite Markers Used in One or Both Linkage Analyses (Xpter to Xqter)

Locus	Primers	Cytogenetic localisation	Reference
DXS996	AFM212xe5	Xp22.33	Weissenbach et al., 1992
DXS237	GMGX9AC	Xp22.32	Gedeon et al., 1992
DXS85	L782	Xp22.31	Chang et al., 1994
DXS16	pSE3.2L	Xp22.31	Chang et al., 1994
DXS999	AFM234yf12	Xp22.13	Weissenbach et al., 1992
DXS365	RX314	Xp22.13	Browne et al., 1992a
DXS989	AFM135xe7	Xp22.12	Weissenbach et al., 1992
DXS451	kQST-80	Xp22.12	Browne et al., 1992a
DXS992	AFM184xg5	Xp21.1-21.3	Weissenbach et al., 1992
DYS	5′DysII	Xp21.1	Feener et al., 1991
DXS1068	AFM238yc11	Xp11.4	Weissenbach et al., 1992
MAOA	MAOA	Xp11.3-11.4	Black et al., 1991
DXS1003	AFM27xf5	Xp11.3	Weissenbach et al., 1992
PFC	PFC-CA	Xp11.23-11.3	Coleman et al., 1991
DXS1126	EAD	Xp11.23	Donnelly et al., 1994b
DXS991	AFM15xf6	Xp11.21	Weissenbach et al., 1992
ALAS2	I7CA	Xp11.21-11.22	Cox et al., 1992
AR	AR(AGC)	Xq12	Donnelly et al., 1994b
PGK1P1	PGK/5	Xq12	Browne et al., 1992b
DXS1125	46AD	Xq12-13.1	Donnelly et al., 1994b
DXS453	Mfd66	Xq12-13.1	Weber et al., 1990
DXS1124	45AD	Xq13.1	Donnelly et al., 1994b
DXS1002	AFM249vh5	Xq21.31	Weissenbach et al., 1992
DXS986	AFM116xg1	Xq21.1	Weissenbach et al., 1992
DXS990	AFM136yc7	Xq21.33	Weissenbach et al., 1992
DXS458	Mfd79	Xq21.33	Weber et al., 1990
DXS454	Mfd72	Xq22.1	Weber et al., 1990
DXS1120	GAD	Xq22.3	Donnelly et al., 1994b
DXS456	XG30B	Xq22.1	Luty et al., 1990
DXS424	XL5A	Xq24	Luty et al., 1990
DXS425	XL90A3	Xq24	Luty et al., 1990
HPRT	HUMHPRTB	Xq26.1	Edwards et al., 1991
DXS984	AFM105xc5	Xq27.1	Weissenbach et al., 1992
DXS297	VK23AC	Xq27.3	Richards et al., 1991
DXS1123	41AD	Xq28	Donnelly et al., 1994b
DXS1108	sDF-2	Xq28	Freije et al., 1992

TABLE III. Two-Point Analysis Between *MRX30* and Marker Loci (Xpter to Xqter)

	θ								
Locus	0.00	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θmax
DXS996	−∞	−7.53	−4.11	−2.70	−1.40	−0.73	−0.31		
DXS85	−∞	−5.84	−3.13	−2.03	−1.03	−0.53	−0.21		
DXS999	−∞	−3.67	−1.69	−0.94	−0.35	−0.15	−0.07		
DXS365	−∞	−3.23	−1.77	−1.16	−0.64	−0.39	−0.20		
DXS989	−∞	−3.49	−2.03	−1.38	−0.75	−0.40	−0.17		
DXS451	−∞	−3.45	−1.94	−1.27	−0.64	−0.33	−0.13		
DYS	−∞	−3.30	−1.82	−1.15	−0.52	−0.21	−0.05		
DXS1068	−∞	−2.73	−1.35	−0.78	−0.29	−0.07	0.01		
MAOA	−∞	−3.40	−1.87	−1.19	−0.53	−0.21	−0.05		
DXS1003	−∞	−4.40	−2.32	−1.45	−0.65	−0.26	−0.06	−0.06	0.4
DXS986	0.76	0.75	0.71	0.65	0.52	0.35	0.18	0.76	0.0
DXS990	−∞	0.74	1.27	1.36	1.19	0.86	0.45	1.36	0.1
DXS458	1.51	1.49	1.41	1.30	1.03	0.72	0.38	1.51	0.0
DXS454	1.64	1.62	1.53	1.40	1.11	0.77	0.40	1.64	0.0
DXS1120	1.88	1.85	1.72	1.54	1.17	0.76	0.32	1.88	0.0
DXS456	1.85	1.82	1.70	1.55	1.20	0.80	0.36	1.85	0.0
DXS424	−∞	0.45	0.99	1.10	0.99	0.71	0.37	1.10	0.1
DXS425	−∞	−1.25	−0.55	−0.27	−0.03	0.05	0.05		
HPRT	−∞	−3.16	−1.71	−1.06	−0.44	−0.14	−0.01		
DXS984	−∞	−3.10	−1.66	−1.02	−0.40	−0.11	0.01		
DXS1123	−∞	−7.48	−4.04	−2.61	−1.27	−0.59	−0.20		
dXS1108	−∞	−3.91	−1.89	−1.08	−0.40	−0.12	−0.02		

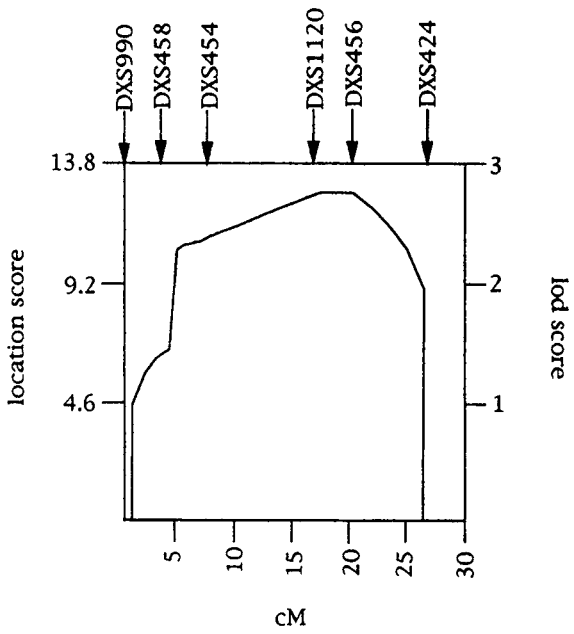

Fig. 3. Placement of *MRX30* into the multipoint background map. The maximum location score was 12.79 at *DXS1120* and *DXS456*, equivalent to a multipoint lod score of 2.78.

whose pattern of expression and perhaps putative function are compatible with a role in brain development. The "seeds" for many such candidate genes are being rapidly produced in the form of ESTs isolated from human brain libraries [see, for example, Adams et al., 1993] and mapped to somatic cell hybrid or radiation cell hybrid panels.

In conclusion, we have determined the regional locations of the genes responsible for X-linked non-specific mental retardation in two families. The localisation for one of these, *MRX30*, has delineated a new region of the X chromosome containing a gene for non-specific X-linked mental retardation. Predictive diagnosis of the carrier status and prenatal diagnosis will now be possible where there are informative flanking markers, especially for *MRX31* where the localisation is within an interval of 12 cM.

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TABLE IV. Two-Point Analysis Between *MRX31* and Marker Loci (Xpter to Xqter)

	θ								
Locus	0.00	0.01	0.05	0.1	0.2	0.3	0.4	Zmax	θmax
DXS237	−∞	−2.24	−0.88	−0.36	0.05	0.16	0.13		
DXS16	−∞	−6.32	−3.53	−2.34	−1.21	−0.60	−0.23		
DXS999	−∞	−5.59	−2.87	−1.77	−0.77	−0.31	−0.07		
DXS365	−∞	−4.09	−2.09	−1.30	−0.57	−0.21	−0.04		
DXS989	−∞	−6.89	−3.49	−2.12	−0.89	−0.32	−0.06		
DXS451	−∞	−3.79	−1.80	−1.03	−0.39	−0.13	−0.02		
DXS992	−∞	−6.42	−3.05	−1.71	−0.56	−0.09	0.07		
DYS	−∞	−6.59	−3.21	−1.86	−0.69	−0.18	0.02		
DXS1068	−∞	−6.30	−2.93	−1.16	−0.48	−0.03	0.10		
MAOA	−∞	−0.34	0.31	0.52	0.60	0.50	0.30		
DXS1003	−∞	−0.61	0.60	0.96	1.05	0.84	0.48		
PFC	−∞	0.62	1.16	1.25	1.10	0.77	0.38		
DXS1126	−∞	1.39	1.88	1.91	1.63	1.15	0.58	1.91	0.1
DXS991	2.06	2.02	1.88	1.69	1.29	0.85	0.40	2.06	0.0
ALAS2	1.45	1.43	1.32	1.18	0.88	0.56	0.24	1.45	0.0
AR	3.44	3.38	3.16	2.86	2.23	1.52	0.76	3.44	0.0
PGK1P1	2.06	2.02	1.88	1.69	1.29	0.85	0.40	2.06	0.0
DXS1125	0.87	0.86	0.81	0.73	0.53	0.32	0.13	0.87	0.0
DXS453	3.31	3.26	3.04	2.76	2.15	1.47	0.73	3.31	0.0
DXS1124	−∞	−1.54	−0.83	−0.52	−0.23	−0.09	−0.02	−0.02	0.4
DXS1002	−∞	0.62	1.16	1.25	1.10	0.77	0.38		
DXS986	−∞	−0.10	0.49	0.63	0.61	0.42	0.18		
DXS454	−∞	−1.38	−0.12	0.30	0.52	0.47	0.29		
DXS1120	1.47	1.47	1.42	1.34	1.12	0.81	0.44		
DXS456	−∞	−0.84	−0.17	0.07	0.22	0.22	0.13		
DXS424	−∞	−2.18	−0.86	−0.36	0.02	0.12	0.10		
DXS425	−∞	−4.60	−1.96	−0.95	−0.15	0.11	0.13		
HPRT	−∞	−5.87	−3.10	−1.95	−0.90	−0.39	−0.12		
DXS984	−∞	−4.09	−2.07	−1.27	−0.57	−0.25	−0.09		
DXS297	−∞	−4.94	−2.82	−1.91	−1.02	−0.53	−0.21		
DXS1123	0.63	0.60	0.48	0.34	0.13	0.02	0.00		
DXS1108	−∞	−0.39	0.21	0.38	0.43	0.34	0.19		

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